On Oxnerella maritima, nov. gen., nov. spec., a New Heliozoon, and Its Method of Division; with Some Remarks on the Centroplast of the Heliozoa.

By

Clifford Dobell,

Imperial College of Science, London, S.W.

With Plate 27.

In 1913 I found a minute and pretty heliozoon in some small tanks of sea-water in which I had been cultivating Foraminifera, Trichosphærium, and other marine Protozoa. For some weeks the organisms multiplied rapidly and became very abundant. I studied them alive as carefully as possible, and made a number of fixed and stained preparations in order to study their method of division in detail. I also made a number of notes and drawings at the time, but was too much occupied with other work to set them in order for publication. As the organisms seem not to have been described hitherto, and as their division presents certain features of interest, I now take the opportunity of putting my observations on record.

1. General Description of the Organisms.

Morphology.—The living organisms are typical sun-animalcules of very small size. They are almost spherical, with numerous filamentar pseudopodia radiating in all directions (see Pl. 27, fig. 1). The pseudopodia are so extremely fine that no internal structure can be made out in them, save

at their somewhat thicker proximal ends. Here it can be seen that each is provided with a very slender axial rod or fibre, which passes into the centre of the animal. Although sometimes visible, with difficulty, in the living organism, the pseudopodial axes are much more easily distinguishable in fixed and stained[1] specimens (see Pl. 27, fig. 2), in which they appear as almost immeasurably fine radiating lines. In short, the pseudopodia appear to be, on a very small scale, axopodia such as are characteristic of most Heliozoa.

The axes of the pseudopodia can be traced (Pl. 27, figs. 1, 2) through a clear area in the centre of the animal to a minute corpuscle—a so-called "central granule"—in which they are rooted. This little body is not always easily seen in living specimens, on account of its very small size, and on account of the many food-bodies present in the surrounding protoplasm. But in stained preparations it is always visible (cf. Pl. 27, fig. 2), and presents various appearances which will be described below. The arrangement of the pseudopodia and "central granule" is similar to that already described in Acanthocystis, Wagnerella, and other Heliozoa.

Many of the pseudopodia of a living specimen are extremely long, attaining a length equal to three or four times the diameter of the animal's body (cf. Pl. 27, fig. 1). In fixed and stained specimens, however, they are usually much contracted, and consequently appear shorter and fewer (Pl. 27, fig. 2). During life they are studded irregularly with numerous minute granules (Pl. 27, fig. 1), which constantly stream up and down them. These streaming granules are already well known in other Heliozoa.

With the exception of the clear area surrounding the

[1] I fixed and stained the organisms in various ways. The best fixation was obtained with Bouin's fluid and Schaudinn's sublimate-alcohol; and by far the best of the stains which I tried was my alcoholic iron-alum hæmatein, which I have described elsewhere (Dobell, 1914). All the figures here reproduced were stained by this method, which for delicacy and detail can hardly be surpassed.

central granule, all the protoplasm of the body is usually closely packed with ingested food-bodies (Pl. 27, fig. 1). The protoplasm itself is colourless. There is no obvious differentiation of the cytoplasm into ectoplasm and endoplasm.

There is no contractile vacuole, no stalk, and no spicular skeleton, either external or internal; nor is a gelatinous investment present.

There is a single relatively large nucleus (Pl. 27, figs. 1, 2), which is vesicular, with a large central karyosome. The nucleus is excentrically placed, and usually ovoid, its more pointed end being directed towards the centre of the organism (Pl. 27, figs. 1, 2). Those pseudopodial axes which lie in contact with the nuclear membrane often stain more deeply than the rest, and thus appear as dark lines traversing the surface of the nucleus. (This can be seen in the nucleus shown in Pl. 27, fig. 7.)

Most specimens are not perfectly spherical, and the ingested food-bodies often project above the surface of the body, giving it an irregular contour. It is thus impossible to measure the diameter of most individuals with great accuracy. The diameter of fifty fixed and stained specimens (all nearly spherical, and not dividing), measured as nearly as possible, showed a range from about 10 μ to 22 μ, the mean being about 14 μ.

2. Systematic Position.

From the foregoing brief account of the structure of this heliozoon, it will be clear that it belongs to the group of naked forms (such as Actinosphærium, etc.) which constitute the order Aphrothoraca of R. Hertwig. Schaudinn (1896) enumerates nine genera in this order, which all differ in important particulars from the present form. Actinolophus, Zooteira, and Wagnerella (= Haeckelina) are stalked; Actinosphærium and Gymnosphæra are multinucleate, and possess sharply differentiated ectoplasm and endoplasm; Actinophrys is uninucleate, but possesses

no central granule; and the pseudopodia of Monobia, Myxastrum, and Camptonema are so different in various ways from those of the form under consideration that it is impossible to place it in any of these genera. There is no genus which combines the characters peculiar to my organisms—namely, pseudopodia with axial fibres rooted in a central granule; a single nucleus; no contractile vacuole; no distinct ectoplasm and endoplasm; no stalk.

It therefore appears necessary to introduce new generic and specific names for the present form, and I propose to call it Oxnerella[1] maritima, n. g., n. sp. It will be seen, I think, that Oxnerella bears much the same relation to Gymnosphæra that Actinophrys does to Actinosphærium; for Actinophrys is like a uninucleate Actinosphærium just as Oxnerella is like a uninucleate Gymnosphæra.

3. Habits, Habitat, Etc.

Of the habits of Oxnerella little need be said, for it does not differ in any important ways from many familiar Heliozoa. A few points are worth noting, however.

As the organisms occurred in cultures, I can only describe their behaviour in these; and it is possible that in nature their habits are different. Nevertheless, they appeared so healthy and normal that I do not suppose the differences can be very great.

The animals often float freely in the water, but display a special predilection for the surface film. Many of them were always to be found also at the bottom of the culture-tanks and on the sides, and in these situations they were generally more or less firmly attached by their pseudopodia to the substratum. When the cultures were well stocked, the organisms could be "fished" with a pipette at all levels, and were found attached to all the objects (algæ, stones, etc.) in

[1] In honour of my friend, Dr. Mieczyslav Oxner, of the Musée Océanographique, Monaco.

the aquaria. Cover-glasses suspended at varying depths in the water by means of cotton threads were generally found to have many animals adherent to them after they had been left for one or two days. As animals such as these are not able to swim in any way, their distribution through the motionless water of a tank is probably brought about somewhat as follows: They lie first of all on the bottom—a position which they always take up when removed from one vessel to another. They then creep along the bottom, and up the sides of the aquarium until they reach the surface film. They crawl along this by means of their pseudopodia, and then, becoming detached, they fall slowly towards the bottom once more, alighting upon any foreign body which they may encounter on the way. In nature, no doubt, they are much more extensively distributed by the movements of the water.

The creeping movements of an Oxnerella may be easily observed on a slide under the microscope. If it is not unduly compressed by a cover-glass, it will begin to move about slowly as soon as it has recovered from the shock of being mounted in the preparation. It progresses by a gentle half-gliding, half-rolling motion, dragging itself along by means of its pseudopodia.

One of the features which first attract attention on closely observing the living animal is the streaming of the minute granules on the pseudopodia—of which mention has already been made. Although this streaming is well known in other Heliozoa, the mechanism by which it is brought about is still unexplained, and the motions of the granules are really very puzzling if observed for any length of time. The "granules" are generally supposed to be little knobs or thickenings of the protoplasm of the pseudopodium—an opinion shared by Schaudinn (1896); but it is not impossible that they are really adherent foreign particles borne along by mucus currents. I have been unable to satisfy myself on this point, either in the case of Oxnerella or of other forms displaying the same phenomenon. I have observed that the "granules" do not all stream in the same direction, even on the same

pseudopodium. They may at times be seen to meet one another when coming from opposite directions, and, after appearing to jostle one another for a moment, travel away in opposite directions—sometimes returning along their former paths, sometimes passing one another. In such circumstances their movements appear curiously purposive, and they remind me of the equally remarkable movements of the spindle-shaped bodies (individual organisms?) in the living network of a Labyrinthula.

Oxnerella, like other Heliozoa, largely uses its pseudopodia for capturing its food. In the aquaria this consisted almost entirely of the swarm-spores of green algæ, which were plentiful. The living animals were generally filled with the bright green bodies of these (Pl. 27, fig. 1), in various stages of digestion, and under a low magnification themselves appeared to be green in consequence.

The water in which Oxnerella occurred was sent to London from Plymouth. But it is quite likely that the organisms are widely distributed and by no means uncommon in the sea, and have hitherto escaped notice on account of their minute size.

4. Division.

My chief reason for describing Oxnerella is to enable me to record and figure in detail its method of multiplication by division. I shall therefore enter into this matter now with some particularity.

The two organs whose behaviour is of special interest in division are the nucleus and the so-called "central granule," to which the axial fibres of the pseudopodia are attached. I shall therefore begin by describing these two structures in the "resting" (i. e. not dividing) organism in greater detail.

The Nucleus.—On account of its somewhat large size and its lack of colour, the nucleus of Oxnerella can usually be made out quite clearly in the living organism. It lies embedded among the bright green food-bodies present in the

cytoplasm (see Pl. 27, fig. 1), and it is therefore sometimes necessary to compress the creature slightly with the cover-glass in order to see its nucleus distinctly. In the living organism the nucleus appears as a relatively large vesicle surrounded by a distinct nuclear membrane, and containing a large central karyosome (Pl. 27, fig. 1). Between the membrane and the karyosome there is a clear zone, in which I have not been able to make out any structure in the fresh state (Pl. 27, fig. 1). But in fixed and stained specimens (Pl. 27, fig. 2) not only can all the structures just described be seen, but in addition the clear zone appears filled with minute chromatin granules supported on an indistinct achromatic network. These appearances are very constant after fixation and staining in various ways, and I incline to the view that the minute granules in the clear zone are actually present—though invisible—in the living organism, and not produced by fixation.

The karyosome appears to be almost homogeneous. It stains uniformly with ordinary chromatin stains, but in iron-hæmatein preparations it usually appears rather paler in the centre than at the periphery (Pl. 27, fig. 2). It contains no granules of any sort.

As already noted, the nucleus is usually oval in outline. It may be noted further that it appears to be rigidly fixed in position. It is not possible to displace it by slight pressure; and even if pressure so great be applied to an organism as to burst it completely and set free most of its enclosed food-bodies, the nucleus still remains behind in the débris of the body. It is probable, I think, that the axial fibres of the pseudopodia which cross the nuclear membrane, and stain more deeply than the rest, are really attached to the nucleus and serve to anchor it in position (see Pl. 27, fig. 7).

The nucleus in the resting state is about 4 μ–5 μ in greatest diameter, ranging from about 3·3 μ to 5·5 μ.

The "Central Granule."—As I shall often have to refer to this important organ in the course of the ensuing descriptions, it seems desirable to give it a name. The term

"central granule" is a periphrasis which does not correctly describe its structure, nor indicate its most important functions; and for brevity, and also for other reasons which will be clear later, I propose to call the organ in question a centroplast.

In a living Oxnerella the centroplast is with some difficulty visible as a minute spherical corpuscle lying at the centre of the organism (Pl. 27, fig. 1). It is rather feebly refringent, and is surrounded by a clear zone of protoplasm quite free from all food-bodies or granules. The size of this zone varies a good deal in different individuals. Radiating from the centroplast and traversing the clear zone in all directions can be seen the central ends of the axial fibres of the pseudopodia.

In fixed and stained specimens all these structures can be studied in greater detail. The centroplast itself (Pl. 27, figs. 2, 5) generally appears as a minute clear sphere with a deeply-staining granule at its centre. The periphery of the sphere stains deeply, as though it were clothed with a very delicate membrane. The axial fibres, when traced through the clear zone of protoplasm towards the centroplast, appear to terminate in minute knobs or granules on its external membrane, and cannot as a rule be traced beyond this to its central granule (Pl. 27, fig. 5).

The central granule of the centroplast stains deeply with iron-hæmatein, less deeply with carmine stains. In its entirety the centroplast thus resembles a tiny nucleus with a karyosome and nuclear membrane.

If one examines with care the centroplasts of a number of different individuals, it can be seen that they do not all present the appearances just described, though these are the most usual. In certain individuals no differentiation into central granule and surrounding membrane can be discovered. The entire centroplast is a minute, deeply-staining, and homogeneous dot (Pl. 27, fig. 3), to which the pseudopodial fibres appear to be attached directly. In other specimens a very minute central granule appears to have become

differentiated in the centre of this minute darkly-staining mass (Pl. 27, fig. 4); and still other specimens show gradual transitions to the "typical" form of centroplast (Pl. 27, fig. 5). Further, organisms can also be found in which the whole centroplast seems to have greatly increased in size (Pl. 27, fig. 6). The central granule is larger, and the clear zone separating it from the membrane has also expanded. In such specimens the axial fibres seem no longer to terminate on the membrane, but to pass through it to the central granule. I believe they are actually attached to this, but from the very small size of all the structures it is hardly possible to be absolutely certain of their relations. This last condition (Pl. 27, fig. 6) affords an easy transition to that first described (Pl. 27, fig. 3).

The appearances just described strongly suggest that cyclical changes occur in the centroplast of the "resting" animal. Similar appearances have already been described and thus interpreted in Wagnerella (Zülzer, 1909). Comparison, moreover, with the cyclical changes described in the centrosome of the metazoan egg (Vejdovský and Mrázek, 1903) at once suggests itself. Although both the interpretation and the comparison appear to me justifiable, I am not completely convinced of their correctness; and I am at a loss to understand what function the "cyclical changes" in Oxnerella can subserve. I thought at one time that they might perhaps play some part preparatory to the division of the organism—for the centroplast has, as will be seen later, an important function in this process. But since the structure of the centroplast is "typical" (Pl. 27, fig. 5) immediately before division and immediately after, this seems to me improbable. There is no obvious reason why it should pass through a cycle of changes—returning finally to its original condition—during the interdivision period.

General Account of the Process of Division.—Before describing in detail the successive phases of division it will be convenient to give a brief general outline of the process.

Division is, in all cases which I have observed, an equal binary fission, resulting in the production of two small organisms exactly like the parent organism in everything save size. Although the organisms which are about to divide are usually of large size, this is not always the case. I have seen a number of quite small forms in various stages of the process. Again, most dividing organisms are filled with food-bodies, but this is not always so. Illustrations of this will be found in the figures.

During division the animal remains at rest, with all its pseudopodia completely retracted. Not until the two daughter-organisms are completely separated do the pseudopodia again make their appearance. I believe, moreover, that the animal is always attached to some substratum during division; that is to say, it does not divide while floating freely in the water. I infer this from the fact that I never obtained any division stages in organisms suspended in the water; whereas I obtained many in the organisms attached to cover-glasses floating on the surface film or placed at various levels in the aquaria. Most of the dividing organisms were found attached to the surface film.

The nucleus divides by mitosis, the centroplast playing the part of a centrosome. The whole process can really be seen at a glance by inspecting the figures (Pl. 27, figs. 8–22), but the following brief account may be given in amplification.

Prophases.—The centroplast is the first organ to divide. Immediately before division it is in the "typical" condition (Pl. 27, fig. 5), displaying a large central granule and a distinct membrane. It then becomes elongated, and the central granule divides into two (Pl. 27, fig. 7). In the early stages of division the central granule appears (see Pl. 27, fig. 7) as a minute cylindrical body with two deeply-stained polar caps—presumably halves of the original granule. A little later, however, the caps become minute spherical granules, connected by a deeply-staining strand (Pl. 27, fig. 8). This appearance is exactly comparable with that of a dividing centrosome, at the stage when the daughter-centrosomes are

united by a centrodesmose. Up to this stage (Pl. 27, fig. 8) the animal remains spherical, with a few pseudopodia still visible. These are now completely retracted, and the animal becomes drawn out into an elongate form. As it does so, the daughter-centroplasts draw further apart, but still remain connected by their "centrodesmose," which occupies the centre of the long axis of the body (Pl. 27, fig. 9). The dividing centroplast at this stage appears, in consequence, as a much attenuated dumb-bell. The centrodesmose is an excessively fine but quite distinct line. Throughout the division of the centroplast the central ends of the axial fibres of the pseudopodia are visible, radiating through the cytoplasm exactly like the astral rays of a centrosome (Pl. 27, figs. 8, 9).

The "centrodesmose" now vanishes, and the two daughter-centroplasts are seen—each surrounded by a few short "astral rays"—to occupy symmetrical positions at opposite ends of the organism.

During the early stages of the division of the centroplast the nucleus increases in size (Pl. 27, fig. 8). As the animal elongates, the nucleus gradually travels towards the middle of the body, until it finally takes up a position midway between the two daughter-centroplasts (Pl. 27, figs. 8–11). During this translocation the nucleus usually has an irregular, misshapen appearance (Pl. 27, figs. 9, 10). Sometimes, also, its karyosome becomes fragmented (Pl. 27, fig. 10). But as soon as it reaches its station between the two centroplasts, it recovers its spherical form and lies as a large and conspicuous vesicle at the very centre of the whole animal (Pl. 27, fig. 11). The karyosome now begins to diminish in size (Pl. 27, figs. 11, 12), and as it does so, the chromatin granules in the rest of the nucleus increase in size and number. These granules, therefore, are probably formed in part at the expense of the karyosome.

During these nuclear stages, the centroplasts also undergo changes. They become more or less elongated in a direction transverse to the long axis of the dividing organism (Pl. 27,

figs. 11, 12). Their central granules thus appear as short rods, sometimes slightly knobbed at the ends.[1] The length of these "rods" is different in different individuals (cf. for instance, Pl. 27, fig. 11, with Pl. 27, fig. 12).

A definite spindle now begins to make its appearance between the centroplasts. At first (Pl. 27, fig. 12) a few spindle-fibres only can be seen, stretching from the centroplasts to the nucleus. They gradually become more conspicuous and numerous, however, until a perfect spindle figure is produced, with the centroplasts at its poles—exactly like typical centrosomes (Pl. 27, figs. 13, 14). Whilst the spindle is forming, the nucleus undergoes further changes. The karyosome disappears; the chromatin granules become fainter, and chromosomes make their appearance arranged transversely across the nucleus (Pl. 27, figs. 12, 13). Meanwhile the nuclear membrane becomes less distinct, and the nucleus becomes drawn out towards the poles of the spindle, as though it were actively pulled by the spindle-fibres of the centroplasts (Pl. 27, figs. 12, 13). Finally, the nuclear membrane vanishes, the chromosomes take up their position on the equatorial plate, and a typical mitotic figure results (Pl. 27, fig. 14).

There are several details which I have not been able to make out with certainty, chiefly on account of the very small size of all the structures concerned. In the first place, the mode of origin of the chromosomes in the nucleus is extremely difficult to ascertain. The appearances are as I have just described them, and the following seems the most plausible interpretation. The chromosomes are formed chiefly (or wholly) from the substance of the karyosome—as in certain amœbæ and other Protozoa (see, for example, Dobell (1914), Jameson (1914), etc.). The "chromatin" granules surrounding the karyosome in the resting nucleus probably disinte-

[1] It is probable, I think, that this appearance should be interpreted, not as a rod with knobbed ends, but as an optical section of a disc with a thickened rim, viewed edgewise. The structure is too small, however, for me to speak with certainty.

grate, and pass on to the spindle-fibres (Pl. 27, figs. 11–14), which are very dark and granular between the "asters" and the equatorial plate (Pl. 27, fig. 14). I have found no spireme stage in the prophases, but am not prepared to deny its occurrence.

I have not succeeded in counting the chromosomes on the equatorial plate. They are extremely small and in the form of short rods so closely packed together (Pl. 27, fig. 14) that their number can only be roughly guessed. As the equatorial plate is probably in the form of a ring, and as rather more than ten chromosomes can as a rule be counted across it when presented edgewise, I estimate the chromosome number as probably about twenty-four.

There are some peculiarities in the spindle which require further notice. At all stages, after it is fully formed, it shows a differentiation into two parts, which do not stain alike. There is, first, an unstained or achromatic central area immediately surrounding the equatorial plate (Pl. 27, fig. 14), so that the whole of the middle part of the spindle may be compared with a tiny clear globe with the chromosome ring forming its equator. Secondly, there is, on either side of the clear central part, a more deeply-stained and granular portion of the spindle which resembles a truncated cone with its apex directed towards the centroplast (Pl. 27, figs. 14, 15). The cones become paler in the region of the centroplasts, and the ends of the spindle-fibres appear to be rooted partly in the central granules of the latter, and partly in their membranes (Pl. 27, fig. 14). There is no sharp demarcation between the achromatic central part of the spindle and the stainable cone-like ends, so that the two parts appear to be differentiations of one and the same structure—the spindle—rather than separate elements. They are, no doubt, respectively homologous with the so-called "polar plates" and "achromatic cones" described in the mitotic figures of Actinosphærium (cf. Brauer (1894), R. Hertwig (1898)).

Metaphase.—The chromosomes on the equatorial plate now divide so that two daughter-plates are formed. It is not

possible to ascertain how the chromosomes divide, on account of their extremely small size. But since the chromosomes on the equatorial plate appear to be short rods (Pl. 27, fig. 14), and those at the metaphase smaller granules (Pl. 27, fig. 15), it seems probable that the chromosomes themselves divide by a transverse constriction into two.

Anaphases.—The daughter-groups of chromosomes now move apart, gradually passing towards the poles of the spindle (Pl. 27, figs. 16, 17, 18). As they do so, they become still more closely packed together, and individual chromosomes can no longer be resolved. In the early anaphases (Pl. 27, fig. 16) distinct spindle-fibres can be seen between the chromosome groups, but later the fibres become less distinct and more irregular (Pl. 27, figs. 17, 18). Simultaneously, the differentiated structure of the spindle becomes less distinct, and finally vanishes.

During the anaphases the centroplasts at the poles of the spindle also undergo certain changes. Their central granules are often much drawn out during the later stages, so that they appear in optical section as fairly long rodlets (Pl. 27, figs. 17, 18). These then shorten, and thicken somewhat, and they then become bent into the form of incomplete rings, open on the side nearest the spindle (Pl. 27, fig. 20). Finally, a closed ring is formed, which becomes converted into a minute, homogeneous, darkly-staining central granule in each daughter-centroplast (Pl. 27, fig. 19). All these changes occur during the late anaphases and early telophases, and they do not always synchronize with the nuclear changes. For example, Pl. 27, fig. 20, shows an earlier stage in the reconstruction of the centroplast than Pl. 27, fig. 19, though the nuclear stage in Pl. 27, fig. 19, is earlier than that in Pl. 27, fig. 20. Centroplast and nucleus are thus independent of one another to some extent at this period. I have found a good deal of variation in this respect in different individuals. In at least one specimen I have seen the centroplasts completely reconstructed, and spindle-fibres no longer visible, before the telophases had begun.

Telophases.—At the end of the anaphase stages each set of chromosomes breaks up into a ball of deeply-staining granules, with which probably a part of the substance of the spindle becomes incorporated to form the daughter-nuclei (Pl. 27, fig. 19). Between these two nuclei the spindle-fibres rapidly disappear (Pl. 27, figs. 19, 20). As reconstruction of the nuclei proceeds, a part of the chromatin becomes aggregated at the centre of each, forming the new karyosomes (Pl. 27, figs. 19, 20). The daughter-nuclei then soon assume the structure characteristic of the ordinary resting nuclei. The stages in this process will be evident from the figures without further description (Pl. 27, figs. 20–22), since they do not differ from the telophases of many other nuclear divisions —for instance, those in Amœba glebæ, which I have elsewhere described (Dobell, 1914).

Constriction of the protoplasmic body of the organism into two begins during the anaphases (cf. Pl. 27, figs. 17, 18), and is completed during the telophases (Pl. 27, figs. 20–22). As the two little daughter-individuals separate they gradually form their pseudopodia, the axes of which can be seen to spring from the new centroplasts (Pl. 27, fig. 22). Each animal very soon acquires the typical form of the adult, with central centroplast, excentric nucleus in close relation to it, and so on. Each daughter-organism also "inherits" about an equal share of the food-masses of its parent.

It is perhaps worthy of note that the final constriction of the body into two is preceded by a stage in which there is a narrow strand of protoplasm connecting the two organisms. (See Pl. 27, fig. 21. In later stages, before complete separation, the strand becomes much finer before it snaps.) This detail in division distinguishes some of the free-living amœbæ —e.g. A. glebæ and related forms—from others, in which the constriction is effected more sharply, without the persistence of a connecting strand (e.g. "A. limax" and related forms).

I have not been able to watch the whole process of division, from beginning to end, in the same living organism. I have

found it impossible to study the dividing nucleus in unstained specimens, on account, probably, of the small size of all the structures concerned. Elongated organisms—which are seen, in stained specimens, to be at any stages of the prophases or metaphase—complete their division in times varying from about five minutes to a quarter of an hour. These are the only living forms which I have been able to recognise as forms about to divide; and they are actually, as will be evident, already somewhat far advanced in the process. On analogy with other organisms, and from the few observations which I have been able to make, I should estimate the total time taken for division at about twenty minutes to half an hour.

5. Some Remarks on the Centroplast of the Heliozoa.

I propose to terminate this account of Oxnerella with a brief consideration of certain problems presented by the centroplast. Although this organ is probably peculiar to a small section of the Heliozoa—for it is not known to be present in any other organisms — a consideration of its functions and homologies leads to some of the fundamental problems in the morphology of the Protozoa.

The facts so far established concerning the centroplast may first be enumerated before their interpretation is discussed. They can, I think, be best reviewed in their historic order.

The centroplast appears to have been first seen in Acanthocystis by Grenacher (1869). He figured it, and described it as "a tiny pale corpuscle" lying at the centre of the organism. He believed, moreover, that the axial fibres of the pseudopodia were rooted to it; but he was unable, as he says, "to prove a direct connexion." The proof was, however, soon furnished by F. E. Schulze (1874), R. Hertwig (1877), and others. All later workers have confirmed their observations, and extended them to all the Heliozoa known to possess centroplasts.

The anatomical relations of the centroplast having been

thus established, it became obvious that at least one function of this organ is skeletal; it serves as a central point of attachment, or focus, for the axial fibres of the radiating pseudopodia. But whether the centroplast possessed any other function was not apparent from its morphological relations, and had, therefore, still to be proved.

About a quarter of a century ago, the centrosome, then recently discovered by E. van Beneden, was occupying the attention of most cytologists. If an Acanthocystis is homologous with a metazoan cell—as it was generally supposed to be—and its nucleus homologous with the cell's nucleus; then if the centrosome is a permanent organ in the cell—as was also then generally believed—there ought clearly to be some corresponding organ in the Acanthocystis. And seemingly there was: there was the centroplast, already described and figured exactly like a centrosome. It was therefore almost inevitable that someone should suggest that centrosome and centroplast are homologous structures.

So far as I am aware, the first suggestion of this homology to appear in print came from Bütschli (1892). It was, however, nothing more than a suggestion based upon the striking structural similarity of the two organs—a similarity which is, as I shall try to show, somewhat misleading.

At about this time a new fact was brought to light by Sassaki. Whilst studying a new heliozoon (Gymnosphæra albida), he discovered that the division of the whole organism is preceded by a division of the centroplast—a fact which justified him, more than a mere structural resemblance could, in homologizing this organ with the centrosome of a metazoan cell. It should be noted that Sassaki's observation had been made and written down by him in 1891,[1] although it was not published until 1894. To the Japanese zoologist, therefore, belongs the credit, not merely of first suggesting that the centroplast is the homologue of a centrosome, but of having

[1] See footnote to Sassaki's paper (p. 45) by R. Hertwig.

discovered an important fact upon which this homology could be based.[1]

Now the heliozoon studied by Sassaki (Gymnosphæra) is multinucleate, though it possesses but a single centroplast; and he was unable to demonstrate that this organ plays the part of a centrosome during the nuclear divisions. To this extent, therefore, the suggested homology of centroplast and centrosome remained unsupported by facts.

This deficiency was soon made good. In a brief but memorable paper, published in 1896, Schaudinn (1896A) described the division of Acanthocystis aculeata, a heliozoon possessing a centroplast and a single nucleus. "From this brief account," he says, "it will, I think, be clear that the nuclear division in the heliozoon investigated takes place in essentially the same way as the typical mitosis of a metazoan cell, and that the central granule [= centroplast] corresponds to the centrosome of the metazoan cell." Schaudinn published only six figures of division stages in Acanthocystis, leaving many gaps which he promised to fill in his full account, which was never published. He stated further that he had observed similar phenomena in Acanthocystis turfacea, A. myriospina, Raphidiophrys, Sphærastrum, and Heterophrys. The division of all these forms is still unknown—or, at least, undescribed; and no full account of the division of Acanthocystis aculeata has ever appeared.

To my knowledge, only one partial confirmation of Schaudinn's statements has hitherto been published. I refer to Zuelzer's description of Wagnerella (1909). Of several different methods of nuclear division described by the authoress in this peculiar heliozoon, there is one in which the centroplast appears to behave like a centrosome.[2] But although

[1] Another Japanese zoologist—Watasé—put forward the same suggestion in 1894. In the same year, also, Heider demonstrated the centroplast of Raphidiophrys—previously described by F. E. Schulze—and emphasized its resemblance to a centrosome. Both these workers, however, knew beforehand of Sassaki's results.

[2] I refer to the multiple division of the "head" of the organism, whereby a brood of young is formed. I do not consider the other

the centroplast appears to divide before the nucleus, and to take up the position of a centrosome during the division of the latter, it is a curious fact—assuming the account to be correct—that the nuclear division itself is apparently a kind of amitosis. These observations do not furnish a very substantial confirmation, therefore, of Schaudinn's statement that the centroplast plays the part of a centrosome in the mitosis of the heliozoan nucleus.

It has long seemed to me desirable that the behaviour of the centroplast during the division of those Heliozoa possessing this organ should be carefully studied and described. As experience has shown, Schaudinn's brief statements and incomplete descriptions are not always to be accepted as established facts; for the magnitude of his mistakes is beginning already to rival that of his successes.

It is for this reason that I have described the division of Oxnerella in some detail. My own observations induce me to believe that Schaudinn's account of the division of Acanthocystis is almost certainly correct; and it therefore appears to me justifiable to conclude that the centroplast actually does behave during nuclear division, in those Heliozoa which possess a single nucleus, precisely like the centrosome in a typical mitosis in a metazoan cell. We have still to learn, however, the part (if any) played by the centroplast in the division of those Heliozoa which are multinucleate.

Although I follow Schaudinn up to a certain point—as just noted—I am unable to concur in his speculations. On the evidence of the observations just considered, but more especially from his observations on the origin of the centro-

---

methods of nuclear division in which a "centriole" is said to be present, and later to become the centroplast of a new individual. Although this is several times stated to occur, I can find no direct evidence in support of the statement, which appears to be merely an assumption based upon Schaudinn's statements about Acanthocystis. Accordingly, I do not think Zuelzer's assertions can be regarded as confirmations of Schaudinn's results.

plast in the young forms of Acanthocystis produced by budding, he was led to advance certain views, now well known, on the phylogeny of the centrosome. These views depend, for their validity, upon the assumptions (1) that his observations on the Heliozoa were correct (those relating to the buds—the most important for his speculations—have never been confirmed); (2) that the centroplast and the centrosome are homologous organs; (3) that the Protozoa, being "primitive animals," furnish us with data for determining the phylogeny of the Metazoa. As regards the first assumption, I would merely note that the observations in question have been confirmed in part only. As regards the third, I will only say that I regard it, as I have elsewhere pointed out (1911) as wholly unjustifiable. Concerning the second assumption I shall here say a few words.

For reasons which I have given elsewhere (1911), I do not regard any single protozoon as the homologue of a metazoan cell. A single individual heliozoon—e. g. an Oxnerella—is, in my view, comparable with a single whole metazoon, and not with one of the cells of which it is composed. An Oxnerella is a whole non-cellular organism, whilst a metazoon is a similar whole organism whose body is differentiated into cells. The one displays a non-cellular, the other a cellular, morphological composition. It follows, therefore, that we have no reason to assume that those organs and structures which are peculiar to the cell must have a morphological counterpart in the individual protozoon.

Now the centroplast of Oxnerella and other similar Heliozoa is not an organ whose activities are limited to a transient phase in the life of the organism—the momentary process of division. It is, on the contrary, a complex structure, permanently present, which plays a skeletal part in the organism as a whole, and in connexion with its organs of locomotion and prehension, and a totally different part as an accessory to the process of division. It plays the part of a centrosome, but it does much more. On the other hand, it seems probable that the centroplast of multinucleate Heliozoa

(e.g. Gymnosphæra) is only skeletal in function, and plays no part in nuclear division. (This has still to be determined, but it is not easy to conceive how a single centroplast could act as centrosome to many different nuclei.) The centroplast of the Heliozoa is thus closely comparable with the blepharoplast of the Mastigophora—an organ permanently subserving a skeletal function to the organs of locomotion (the flagella), and in some forms assuming the office of centrosome at division, in others playing no part in this process (as in some trichomonads and in Copromonas respectively, as I have shown in two earlier papers (1909 and 1908)). To say that either the centroplast or the blepharoplast is the homologue of the metazoan centrosome, and to apply the same term to all these structures, appears to me, therefore, inadvisable. By giving so wide a connotation to the term "centrosome" we shall effect an economy in words; but we shall introduce a confusion of thought by grouping together, as the same, certain structures which are in many ways radically different. In the language of the older morphology, I would say that the centroplast and the centrosome may be analogous, but are not homologous, organs.

For these reasons, in addition to those based on general grounds, I consider that all phylogenetic speculations regarding the centrosome, based on a comparison of the Heliozoa—or any other Protozoa—with the cells of Metazoa, should be viewed with considerable scepticism. Opinions on such matters differ, and always will differ; but for my own part, such views as those of Schaudinn, and his many followers, on the "phylogeny of the centrosome," appear to be mere speculations which are not only improperly called "theories," but are even outside the limits of legitimate hypothesis.

6. Diagnosis of Oxnerella maritima.

In conclusion I will add a brief diagnosis of Oxnerella, which will serve at the same time as a summary of the facts recorded in previous pages.

Oxnerella maritima nov. gen., nov. spec. Aphrotho-

racan heliozoon of very small size (10 μ to 22 μ in diameter); spherical, with numerous very fine radiate pseudopodia (axopodia) with streaming granules and with axes rooted in a centrally placed centroplast; no stalk, no contractile vacuole, no gelatinous investment, no spicular skeleton, no sharply-differentiated ectoplasm and endoplasm. Nucleus single, large, excentric; vesicular, with large central karyosome. Reproduction by equal binary fission, in which nucleus divides by mitosis, the centroplast playing the part of a centrosome. Free-floating or creeping; solitary; marine. Food chiefly vegetable matter (in cultures, swarm-spores of green algæ).

References.

Brauer, A. (1894).—"Ueber die Encystirung von Actinosphærium eichhorni Ehrbg.," 'Zeitschr. wiss. Zool.,' vol. lviii, p. 189.

Bütschli, O. (1892).—"Ueber die sogenannten Centralkörper der Zelle und ihre Bedeutung," 'Verhandl. naturhist.-med. Ver. Heidelberg,' vol. iv (N.F.), p. 535.

Dobell, C. (1908).—"The Structure and Life-history of Copromonas subtilis, n. g., n. sp.," 'Quart. Journ. Micr. Sci.,' vol. 52, p. 75.

——— (1909).—"Researches on the Intestinal Protozoa of Frogs and Toads," ibid., vol. 53, p. 201.

——— (1911).—"The Principles of Protistology," 'Arch. f. Protistenk., vol. xxiii, p. 269.

——— (1914).—"Cytological Studies on Three Species of Amœba," ibid., vol. xxxiv, p. 139.

Grenacher, H. (1869).—"Bemerkungen über Acanthocystis viridis, Ehrbg. sp.," 'Zeitschr. wiss. Zool.,' vol. xix, p. 289.

Heider, K. (1894).—Demonstration of Sections of Raphidiophrys, 'Verhandl. deutsch. Zool. Ges.,' vol. iv, p. 94.

Hertwig, R. (1877).—"Studien über Rhizopoden," 'Jena. Zeitschr., vol. xi, p. 324.

——— (1898).—"Ueber Kerntheilung, Richtungskörperbildung und Befruchtung von Actinosphærium eichhorni," 'Abh. bayr. Akad. Wiss.,' vol. xix.

Jameson, A. P. (1914).—"A New Phytoflagellate (Parapolytoma satura, n, g., n. sp.) and Its Method of Nuclear Division," 'Arch. f. Protistenk.,' vol. xxxiii, p. 21.

Sassaki, C. (1894).—"Untersuchungen über Gymnosphæra albida, eine neue marine Heliozoe," 'Jena. Zeitschr., vol. xviii, p. 45.

Schaudinn, F. (1896).—"Heliozoa," in 'Das Tierreich' (Berlin).

——— (1896A).—"Ueber das Zentralkorn der Heliozoen, ein Beitrag zur Centrosomenfrage," 'Verhandl. deutsch. zool. Ges.' (Bonn), p. 113.

Schulze, F. E. (1874).—"Rhizopodenstudien II," 'Arch. mik. Anat.,' vol. x, p. 377.

Vejdovský, F., and Mrázek, A. (1903).—"Umbildung des Cytoplasma während der Befruchtung und Zelltheilung,', ibid., vol. lxii, p. 431.

Watasé, S. (1894).—"Origin of the Centrosome," 'Biol. Lect., Wood's Holl.'

Zuelzer, M. (1909).—"Bau und Entwicklung von Wagnerella borealis, Mereschk.,' 'Arch. f. Protistenk.,' vol, xvii, p. 135.

EXPLANATION OF PLATE 27,

Illustrating Mr. Clifford Dobell's paper on "Oxnerella maritima, nov. gen., nov. spec., a New Heliozoon, and Its Method of Division; with Some Remarks on the Centroplast of the Heliozoa."

[All figures depict Oxnerella maritima, n. g., n. sp., and are drawn to the same magnification, which is approximately 1500 diameters. Except Fig. 1, all are drawn from whole specimens fixed in Bouin's fluid and stained with alcoholic iron-alum-hæmatein. They were drawn under a Leitz 2 mm. apochromatic objective (N.A. = 1·40), with compensating oculars, using a Leitz achromatic condenser (N.A. = 1·40) with critical illumination.]

PLATE 27.

Fig. 1.—Living organism, slightly compressed to show internal structure. (The rounded masses in the cytoplasm in this and following figures are ingested swarm-spores of green algæ, in various stages of digestion.)

Fig. 2.—Similar specimen, fixed and stained.

Figs. 3–6.—Central region in different individuals, showing various appearances presented by the centroplast.

Fig. 7.—Central region and nucleus of an organism at a very early stage of division of the centroplast. Note the attachment of the nucleus to it by three of the axopodial rays.

Figs. 8–22.—Successive stages in division.

Figs. 8 and 9.—Division of centroplast.

Figs. 10–13.—Prophases.

Fig. 14.—Stage of equatorial plate.

Fig. 15.—Metaphase.

Figs. 16–18.—Anaphases.

Figs. 19–21.—Telophases.

Fig. 22.—Division complete, the two daughter-organisms completely reconstructed.

(In Figs. 15, 16, and 19 the nucleus and surrounding protoplasm only are shown.)